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33CLAIMS:

1. (Amended) A substantially pure DNA fragment encoding an alpha subunit of the human neuronal nicotinic acetylcholine receptor.

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2. A DNA according to Claim 1 wherein said DNA encodes the human alpha2 or alpha3 subunit.

3. A DNA according to Claim 2 wherein said alpha2 subunit is represented by the restriction map set forth in Figure 1, or a DNA fragment having substantial sequence homology with said DNA.

4. A DNA according to Claim 2 wherein said alpha3 subunit is represented by the restriction map set forth in Figure 2, or a DNA fragment having substantial sequence homology with said DNA.

5. (Amended) A substantially pure DNA fragment encoding a beta subunit of the human neuronal nicotinic acetylcholine receptor.

6. A DNA according to Claim 5 wherein said DNA encodes the human beta2 subunit.

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7. A DNA according to Claim 6 wherein said DNA is represented by the restriction map set forth in Figure 3, or a DNA fragment having substantial sequence homology with said DNA.

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8. (Amended) A substantially pure protein encoded by the DNA of any one of Claims 3, 4 or 7.

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9. (Amended) An mRNA encoded by the DNA of any one of Claim 3, 4, or 7.

10. (Amended) Cells transformed with any one of or
5 more than one of the DNA fragments of Claim 1 or Claim 5.

11. (Amended) Cells according to Claim 10 wherein
said cells are also transformed with at least one
additional substantially pure DNA fragment encoding an
10 alpha or a beta subunit of the human neuronal nicotinic
acetylcholine receptor, whereby the cells contain at least
one substantially pure DNA fragment encoding an alpha
subunit of the human neuronal nicotinic acetylcholine
receptor and at least one substantially pure DNA fragment
15 encoding a beta subunit of the human neuronal nicotinic
acetylcholine receptor.

12. Cells according to Claim 11 wherein said DNA
encoding an alpha subunit of the human neuronal nicotinic
20 acetylcholine receptor is selected from the human alpha2
subunit or the human alpha3 subunit, and said DNA encoding
a beta subunit of the human neuronal nicotinic
acetylcholine receptor is the human beta2 subunit.

13. Cells according to Claim 12 wherein said alpha2
subunit is represented by the restriction map set forth in
Figure 1, said alpha3 subunit is represented by the
restriction map set forth in Figure 2, and said beta2
subunit is represented by the restriction map set forth in
30 Figure 3.

14. Cells according to Claim 11 wherein said cells
are eukaryotic cells.

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15. Cells according to Claim 14 wherein said eukaryotic cells are selected from yeast or mammalian cells.

16. Cells according to Claim 15 wherein said yeast
5 cells are selected from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Candida tropicalis* or *Hansenula polymorpha*.

17. Cells according to Claim 15 wherein said
10 mammalian cells are selected from human, rat or mouse cells.

18. (Amended) Cells transformed with any one or more
mRNAs encoding an alpha subunit of the human neuronal
nicotinic acetylcholine receptor or any one or more mRNAs
15 encoding a beta subunit of the human neuronal nicotinic
acetylcholine receptor.

19. (Amended) Cells according to Claim 18 wherein
said cells are also transformed with at least one
20 additional mRNA encoding an alpha or a beta subunit of the
human neuronal nicotinic acetylcholine receptor, whereby
said cells contain at least one mRNA encoding an alpha
subunit of the human neuronal nicotinic acetylcholine
receptor and at least one mRNA encoding a beta subunit of
25 the human neuronal nicotinic acetylcholine receptor.

20. (Amended) Cells according to Claim 19 wherein said
alpha subunit of the human neuronal nicotinic acetylcholine
receptor is selected from the human alpha2 subunit or the
30 human alpha3 subunit, and said beta subunit of the human
neuronal nicotinic acetylcholine receptor is a beta2
subunit.

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21. (Amended) Cells according to Claim 20 wherein said alpha2 subunit is encoded by DNA having the restriction map set forth in Figure 1, said alpha3 subunit is encoded by DNA having the restriction map set forth in Figure 2, and said beta2 subunit is encoded by DNA having the restriction map set forth in Figure 3.

22. Cells according to Claim 19 wherein said cells are amphibian cells.

23. Cells according to Claim 22 wherein said amphibian cells are *Xenopus* oocytes.

24. (Amended) Cells according to Claim 11 further comprising a reporter gene expression construct, wherein said construct contains:

a transcriptional control element; and
a reporter gene encoding a transcription and/or translational product; wherein said transcription control element, in said cell, is responsive to an intracellular condition that occurs when the human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor; said product is, directly or indirectly, detectable; and said gene is in operative association with said transcriptional control element.

25. (Amended) Cells according to Claim 24 wherein said reporter gene construct contains the c-fos promoter and the bacterial chloramphenicol transferase (CAT) gene.

26. (Amended) Cells according to Claim 25 wherein said reporter gene expression construct is included in the plasmid pFC4.

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27. A substantially pure human neuronal nicotinic acetylcholine receptor comprising at least one human alpha receptor subunit and at least one human beta subunit.

5 28. (Amended) The receptor according to Claim 27 wherein said human alpha receptor subunit is an alpha2 or alpha3 subunit and said human beta receptor subunit is a beta2 subunit.

10 29. (Amended) A substantially pure human neuronal nicotinic acetylcholine receptor according to Claim 28, wherein said at least one human alpha receptor subunit is encoded by DNA having the restriction map set forth in Figure 1 (alpha2) or Figure 2 (alpha3), and wherein said at
15 least one human beta receptor subunit is encoded by DNA having the restriction map set forth in Figure 3.

20 30. (Amended) Method for assaying cells for the presence of neuronal nicotinic acetylcholine receptor activity, comprising determining the effect of known neuronal nicotinic acetylcholine agonists and antagonists on the influx of ⁸⁶Rb ions into said cells, relative to the rate of influx of ⁸⁶Rb ions into positive and/or negative control cells.

25 31. A method according to Claim 30 wherein said positive control cells are selected from cells known to express neuronal nicotinic acetylcholine receptors, and said negative control cells do not express nicotinic
30 acetylcholine receptor.

35 32. A method for determining whether a compound has agonist or antagonist activity relative to neuronal nicotinic acetylcholine receptors, said method comprising determining

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the effect of said compound on the influx of ^{86}Rb ions into cells known to express neuronal nicotinic acetylcholine receptors, relative to the rate of influx of ^{86}Rb ions into such cells contacted with positive and negative control compounds.

33. Method for making cells having neuronal nicotinic acetylcholine receptor activity, said method comprising:

(a) transfecting eukaryotic cells with DNA encoding at least one alpha subunit of a neuronal nicotinic acetylcholine receptor and at least one beta subunit of a neuronal nicotinic acetylcholine receptor,

(b) analyzing said transfected cells for the presence of alpha and beta subunit RNAs,

(c) analyzing those cells which are positive for the presence of alpha and beta subunit RNAs for their ability to bind nicotine or a nicotine agonist, relative to the nicotine binding ability of control cells known to produce neuronal nicotinic acetylcholine receptors, and/or control cells known not to produce neuronal nicotinic acetylcholine receptors; and

(d) determining the effect of known neuronal nicotinic acetylcholine agonists and/or antagonists on cells identified according to step (c) as having the ability to bind nicotine or nicotine agonist on the influx of ^{86}Rb ions into said cells, relative to the rate of influx of ^{86}Rb ions into control cells.

34. (Amended) A method according to Claim 33 wherein said positive control cells are selected from cells known to express neuronal nicotinic acetylcholine receptors, and said negative control cells are identical to said eukaryotic cells but which are not transfected with DNA encoding neuronal nicotinic acetylcholine receptor subunits.

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35. Method according to Claim 33 wherein said cells are further screened by taking electrophysiological measurements on whole cells following transfection.

5 36. (Amended) Method according to Claim 33 wherein said DNA further comprises:

a transcriptional control element; and

a reporter gene encoding a transcription and/or translational product; wherein said transcription control
10 element, in said cell, is responsive to an intracellular condition that occurs when the human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor; said product can be, directly or indirectly,
15 detected; and said gene is in operative association with said transcriptional control element.

37. Method for assaying cells for the presence of neuronal nicotinic acetylcholine receptor activity, said
20 method comprising:

(a) analyzing said cells for the presence of alpha and beta subunit RNAs,

(b) analyzing those cells which are positive for the presence of alpha and beta subunit RNAs for their ability
25 to bind nicotine or a nicotine agonist, relative to the nicotine binding ability of control cells known to produce neuronal nicotinic acetylcholine receptors, and/or control cells known not to produce neuronal nicotinic receptors, and

30 (c) determining the effect of known neuronal nicotinic acetylcholine agonists and/or antagonists on cells having the ability to bind nicotine or nicotine agonist on the influx of ⁸⁶Rb ions into said cells, relative to the rate of influx of ⁸⁶Rb ions into positive and/or
35 negative control cells.

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38. A method according to Claim 37 wherein said positive control cells are selected from cells known to express neuronal nicotinic acetylcholine receptors, and said negative control cells do not express nicotinic acetylcholine receptor.

39. A method according to Claim 33 or 37 wherein mRNA from cells which are positive for alpha and beta neuronal nicotinic acetylcholine subunits is injected into oocytes, which are then assayed for the presence of functional neuronal nicotinic acetylcholine receptors.

40. A method according to Claim 37 wherein said cells are further analyzed by taking electrophysiological measurements on whole cells.

41. (Amended) A method according to Claim 37 wherein said cells further contain a reporter gene expression construct comprising:

20 a transcriptional control element; and
a reporter gene encoding a transcription and/or translational product; wherein said transcription control element, in said cell, is responsive to an intracellular condition that occurs when the human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor; said product can be, directly or indirectly, detected; and said gene is in operative association with said transcriptional control element.

30 42. A method for determining whether a compound has agonist or antagonist activity relative to neuronal nicotinic acetylcholine receptors, said method comprising measuring the

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response of cells having neuronal nicotinic acetylcholine receptor activity, when such cells are contacted with said compound, relative to the response of said cells to positive and/or negative control compounds.

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43. (Amended) A method according to Claim 42 wherein said cells having neuronal nicotinic acetylcholine receptor activity contain at least one substantially pure DNA fragment encoding an alpha subunit of the neuronal
10 nicotinic acetylcholine receptor and at least one beta subunit of the neuronal nicotinic acetylcholine receptor.

44. A method according to Claim 43 wherein said positive control compounds are selected from compounds
15 known to elicit a response when contacted with neuronal nicotinic acetylcholine receptors, and said negative control compounds do not elicit a response when contacted with neuronal nicotinic acetylcholine receptors.

20 45. A method according to any one of Claims 31, 34 or 38 wherein said positive control cells are selected from PC12 or IMR32 cells.

25 46. A method according to Claim 42 wherein said response is selected from:

nicotine binding,

⁸⁶Rb ion-flux,

the electrophysiological response of said cells,

or

30 the electrophysiological response of oocytes transfected with RNA from said cells.

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47. (Amended) A method according to Claim 46 wherein said cells further comprise a reporter gene expression construct containing a transcriptional control element; and a reporter gene encoding a transcription and/or translational product, wherein: said transcription control element, in said cell, is responsive to an intracellular condition that occurs when the human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor; and said product is directly or indirectly, readily measured; and wherein said gene is in operative association with said transcriptional control element.

48. (Amended) A method according to any one of Claims 36, 41 or 47 wherein said reporter gene expression construct contains the construct c-fos-CAT.

49. (Amended) A method according to Claim 48 wherein the c-fos-CAT construct is encoded by the plasmid pFC4.

50. A method according to Claim 47 wherein said response is an increase in the amount of said transcription and/or translational product.

51. (Amended) A method according to any one of Claims 30, 32, 36, 37, or 50, wherein said receptors are human neuronal nicotinic acetylcholine receptors.

52. (Amended) A method for identifying DNA fragments encoding alpha or beta subunits of neuronal nicotinic acetylcholine receptors, said method comprising probing a cDNA library or a genomic library with the DNA fragment of Claim 3, 4 or 7, and recovering from said library DNA fragments having a significant degree of homology relative to said DNA fragment.

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